cross-linked by tTG, pellets incubated in BP with and without load were extracted in SDS sample buffer. Extracts were resolved by SDS-PAGE and biotinylated proteins were visualized by western blotting using HRP-streptavidin and chemiluminescence.

Results: FC incorporation into ECM was significantly increased (p=0.001) by mechanical compression compared with incorporation in pellets at rest. Fluid movement alone did not increase FC incorporation over resting levels. Increased BP incorporation was evident in extracts of mechanically loaded pellets as observed by western blotting. One prominent protein band migrating at approximately 150kDa showed two-fold incorporation of BP (p<.05). Other cross-linked proteins were evident, including a pair of protein bands of approximately 45-50 kDa. Streptavidin-reactive bands were also labeled by an antibody to the isopeptide bond specific to transglutaminase activity.

Conclusion: tTG is present in the ECM of articular cartilage and can be regulated by GTP (Summey *et al.*, 2001). We now demonstrate that transglutaminase activity is present in the ECM of porcine neo-cartilage and is increased during cyclic compressive load. The TGase activity was selective for a subset of proteins including a *45-50* kDa and a 150 kDa protein. Cross-linking of specific endogenous matrix proteins by tTG may play a role in strengthening the cartilage matrix in response to mechanical load.

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PA41

TRAUMATIZED KNEE JOINT SYNOVIAL FLUID FAILS TO PROVIDE BOUNDARY LUBRICATION AMONG EMERGENCY DEPARTMENT PATIENTS

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Objective: Chondroprotection of articulation joint surfaces is provided by lubricin, a mucinous glycoprotein, which is a product of megakaryocyte stimulating factor gene expression. Loss of synovial fluid's lubricating ability has been suspected in the pathogenesis of degenerative joint disease. Lubrication by synovial fluid is independent of viscosity and is termed boundary lubrication. This special biological activity in synovial fluid from Emergency Department (ED) patients with traumatized joints was investigated in this study.

Participants: Adult and pediatric patients presenting with radiographically negative knee joint trauma and clinical evidence of joint effusion.
Design: Prospective ED observational study.

Methods: Affected knee joints were aspirated via the lateral aspect with an 18 gauge needle using sterile technique. ED synovial fluid (EDS) aliquots were centrifuged at 12,000g and stored at - 20^0C. Lubrication ability was assayed *in vitro* in an arthrotripsometer oscillating latex opposed to polished glass under a load of 0.35 x 10^6 N/m^2. Results were reported as the coefficient of friction (λ). Control synovial fluid (CSF) aliquots were obtained post-mortem from subjects without joint trauma.

Results: EDS aliquots lubricated poorly with $\lambda = 0.067 \pm 0.056$ SD (N=59) compared with CFS with $\lambda = 0.033 \pm 0.013$ SD ($F(58,4) = 17.3$, $p = 0.006$). The EDS cell count 95% CI was (1287 - 54,209 cells/mm^3). CSF cell counts were negligible. A correlation between EDS cell count and lack of lubricating ability was noted ($r = 0.27$; $p = 0.023$). Only 23.7% of the ED aspirates possessed normal lubricating ability.

Conclusion: Traumatized knee joints, commonly encountered in the ED, appear to be non-lubricated bearings. What role, if any, this plays in delayed cartilage destruction is the subject of an ongoing investigation.

PA42

LONG-TERM EFFECTS OF NONSTEROIDAL ANTINFLAMMATORY DRUGS ON HUMAN CHONDROCYTES IN ALGINATE BEADS

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Objectives: This study was designed to compare the long-term effects (12 days) of nonsteroidal anti-inflammatory drugs (NSAID) on the metabolism of human chondrocytes cultured in alginate beads.

Methods: Enzymatically isolated osteoarthritic (OA) chondrocytes were cultured in alginate beads in a well-defined culture medium (DMEM +ITS+) for 12 days. Interleukin-6 and -8 (IL-6, IL-8), stromelysin (MMP 3) and aggrecan (AGG) productions were assayed by specific enzyme amplified sensitivity immunoassays (EASIA), and prostaglandin E_2 (PGE_2) production by a specific radioimmunoassay. All NSAID were tested at the mean peak plasmatic concentration (Cmax) obtained after oral administration of a therapeutic dose. The Cmax used in this study were 7.5 microg/ml for aceclofenac (ACECLO), 1.4 microg/ml for diclofenac (DICLO), 2 microg/ml for indomethacin (INDO), 3 microg/ml for nimesulide (NIM), 1 microg/ml for rofecocib (ROFE), 0.7 microg/ml for celecoxib (CELE), 7 microg/ml for piroxicam (PIROX), and 25 microg/ml for ibuprofen (IBUP).

Results: At the therapeutic concentration, all NSAIDs tested fully blocked PGE_2 production. Interestingly, ACECLO, DICLO, INDO, NIM and IBUP significantly inhibited both basal and IL-1 beta-stimulated IL-6 production, whereas ROFE, CELE and PIROX had no significant effects. No NSAID showed significant effects on basal and IL-l beta-stimulated IL-8 production, excepted CELE and IBUP which slightly increased basal IL-8 production. ACECLO and INDO increased by *25%* AGG content in the alginate beads, while the other NSAID were without significant effect. Furthermore, no NSAID was able to modify the inhibitory effect of IL-1 beta on AGG production. Finally, NSAID did not modify MMP-3 production.

Discussion From this study, we can conclude that the mechanism of action of NSAIDs seem to be multifactorial and not limited to the inhibition of cyclooxygenases. Furthermore, in our culture conditions, at the and by comparison with other NSAID ACECLO and INDO show a advantageous profile of activity. They fully block PGE_2 production, inhibit IL-6 synthesis and increase aggrecan synthesis. These effects would appear to be advantageous for the long-term treatment of chronic joint diseases such as osteoarthritis.

PA43

UPREGULATION OF BMP2 AND 4 IN CHONDROCYTES OF OSTEOARTFIRITIC LESIONS IN STR MICE

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Aim: The aim of this study was the evaluate the expression of TGF beta 1, 2 and 3 and BMP2, 4 and 6 in spontaneous osteoarthritis of STR/1n mice.